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RESEARCH ARTICLE

EFFECT OF DIETARY CRUDE PROTEIN ON AMMONIA EMISSION, BLOOD PROFILE AND PRODUCTION PERFORMANCE OF BROILER CHICKENS

M.M. Hossain, M.I. Hossain, T. Sultan and L.Y. Asad

Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

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Abstract

The study investigates the relationship between dietary factors, ammonia emission, blood profile, and overall production performance in broiler chickens to optimize efficiency and meet the global demand for chicken meat. A total of 234 Day-Old Lohman Meat broiler chicks were randomly divided into three dietary treatments: high CP (23% in starter, 22% in grower), medium CP (21% in starter, 20% in grower), and low CP (19% in starter, 18% in grower) with three replicates per treatment and 26 birds in each replicate. The results showed significant differences in body weight gain and feed consumption but not in feed conversion ratio over four weeks, with the low CP group exhibiting the lowest ammonia emissions. The dressing percentage and carcass weight, except for thigh weight, varied significantly ($P < 0.05$) among treatments, with the high CP group exhibiting the highest weights in the breast, wing, back, and drumstick. Blood parameters, including haemoglobin, WBC, RBC, neutrophils, and lymphocytes were affected but no significant differences ($P > 0.05$) were observed in platelet count, monocyte count, eosinophil count, PCV and MCV across treatments. The economic impact analysis showed significant differences ($P < 0.05$) in total expenditure and receipts per bird, with the high CP group showing higher values. However, no significant variations ($P > 0.05$) in profit per bird or benefit-cost ratio were observed among the treatments. To maximize benefits from dietary crude protein utilization and reduce ammonia levels, broilers can be fed diets with lower crude protein content, specifically 19% in starter and 18% in grower stages.

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Introduction:-

Poultry farming has emerged as one of the fastest-growing agribusiness industries worldwide. Research on global meat production indicates that poultry is the fastest-growing sector, particularly in developing countries. It is crucial for the poultry industry to benefit from inexpensive, highly efficient, and safe diets. One challenge faced by the industry is the microbial degradation of poultry waste, which produces ammonia (NH_3) and can reduce poultry performance. Ammonia volatilization originates from nitrogen components such as uric acid, urea, and undigested protein. Factors influencing this process include the dry matter and acidity of the excreta, as well as the temperature in the poultry house (Leenstra and Pit, 1990; Nahm, 2003).

Corresponding Author:-Md. Imran Hossain

Address:-Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

Extensive research has explored various methods to control ammonia, such as ensuring adequate airflow, careful litter management (Reece et al., 1979; Hartung and Phillips, 1994), and adding chemicals to the litter like formaldehyde (Veloet et al., 1974) and zeolites (Nakae et al., 1981). While these methods can be effective, they impose additional costs on producers. Reducing nitrogen emissions through dietary management is the most efficient and cost-effective method for controlling ammonia production. This can be achieved by lowering the nitrogen content of the diet. The inclusion of low crude protein (CP) content in poultry diets is one of the most effective strategies to reduce ammonia emissions from poultry waste (Belloiret et al., 2017; Barekatinet al., 2018).

However, low CP diets can negatively impact growth performance and carcass yield in broilers (Bregendahl et al., 2002; Liu et al., 2017; Allameh and Toghyani, 2019). This negative effect is often attributed to protein deficiency (Yu et al., 2019). Consequently, nutritionists have developed strategies to mitigate the adverse effects of low CP diets on broiler growth performance (Kermaniet al., 2017; Goodarzi et al., 2018). Ensuring an adequate supply of amino acids is essential, so diets based on birds' amino acid needs rather than CP content can lower nitrogen excretion by reducing total nitrogen intake (NRC, 1994). Minimizing excess dietary amino acids has been shown to improve broiler performance (Waldroup et al., 2005). Improvements in nitrogen utilization, such as reductions in plasma uric acid, dietary nitrogen intake, excretion and retention, without negatively affecting live performance, have been observed when feeding broilers low-protein diets (Corzo et al., 2004). Therefore, the current study aimed to determine the effects of dietary crude protein levels on ammonia emission, blood profile, and production performance of broiler chickens.

Materials and Methods:-

Experimental design

This study was conducted at a poultry farm located at Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. A completely randomized design was applied in this study by using different concentration levels of crude protein (CP) to assess the ammonia emission, blood profile, and production performance of broiler chickens. A total of 234 broiler chickens were randomly allocated to three dietary CP levels (high, medium, and low) with three replicates per treatment. Each replicate consisted of 26 birds. The allocation of birds to treatments was performed using a random number generator, ensuring that each bird had an equal chance of being assigned to any treatment group.

Birds, housing and management

A total of 234 day-old Lohman Meat broiler chicks with an average body weight of 42 ± 0.2 g were collected from a commercial hatchery and randomly allotted to 9 floor pens, with 26 birds per pen. Rice husk was used as a litter material to keep the floor free from moisture. Just after the arrival of day-old chicks to the poultry farm, the initial weight of the chicks was recorded by a digital electronic balance, vaccination was done, and they were distributed under the hover for brooding. Beginning at one day of age, the chicks were housed at a temperature of 33°C, which was maintained for the first week. When the chicks were one week old, the temperature was reduced by 4°F (2°C) each week until the final temperature of 27°C was reached. Lighting for one-day-old birds was maintained at 22 hours per day for the first two days at 20 lux intensity, with 2 hours of darkness. From the third day, 18 hours of light and 6 hours of darkness were provided so that the chickens could gradually get accustomed to the dark periods. Each room was mechanically ventilated to eliminate the heat, moisture, carbon dioxide, and ammonia from the birds and ensure a proper environment. The composition of starter diets (1-14 days) has a CP value of 23% (high), 21% (medium), 19% (low), and grower diets (15-28 days) have a CP of 22% (high), 20% (medium), and 18% (low). Crumble feed was used as starter (0-2 weeks) and pellet feed for grower (3-4 weeks) rations. Ad libitum feeding and fresh, clean drinking water provided for the rapid growth of broiler chicks up to the end of the four weeks. Left-over feeds and water were recorded to calculate the actual intake. Feeder and drinker sizes were changed according to the age of the birds. Detail composition of feed are presented in table 1 and 2.

Ammonia assessment procedure

A commercially available ammonia test kit, micro-essential hydron ammonia meter tester paper, was used to assess the ammonia level in the broiler house. Ammonia in the air was detected using test strips from the kit. For this purpose, a 1-inch strip of paper was torn off and moistened with 1 or 2 drops of distilled water. Excess water was shaken off the paper, exposed to the air, and tested for 15 seconds. The resulting colour change was then compared with the colour chart provided in the test kit. If the colour changes matched, the data were recorded.

Recorded parameters

After 28 days of rearing and feeding broiler chicken, data were collected for the following parameters: feed intake (g), live weight (g), feed conversion ratio, dressing percentage, carcass weight, and blood parameters (haemoglobin, WBC, RBC, lymphocyte, monocyte, neutrophil, eosinophil, PCV, and MCV). Additionally, profit per bird (Tk) and benefit-cost ratio (total income/total cost of production) were recorded.

Statistical analysis

All data were analyzed using SPSS 24.0 (SPSS Inc., USA). Significant differences among treatments were tested using a one-way analysis of variance (ANOVA) followed by a Duncan multiple comparison test. The level of statistical significance was set at $P < 0.05$ with the standard error of the means.

Results And Discussion:-

Ammonia gas emissions

The NH_3 emission rates during the 28-day rearing period of birds were investigated across varying concentrations of dietary crude protein (CP), as detailed in Table 3. The findings reveal a notable correlation between lower dietary CP levels and diminished NH_3 emissions. In the initial week, average NH_3 levels in both treated and untreated groups exhibited no significant differences ($P > 0.05$). However, statistical significance ($P < 0.05$) emerged during the subsequent 2nd, 3rd and 4th weeks of the rearing period. The high CP treated group (T_1) recorded the highest NH_3 level at 11.63 ± 0.30 ppm, followed by 10.36 ± 0.45 ppm in T_2 and 6.53 ± 0.25 ppm in T_3 respectively. These results align with earlier research by Hernandez et al. (2012), which observed similar trends, affirming that a reduction in dietary crude protein (CP) in broiler diets positively influences nitrogen (N) excretion. Jacob et al. (1994) further supported this result by demonstrating that nitrogen excretion in poultry waste can be reduced by up to 21% through a 2.5% reduction in dietary CP content, supplemented with synthetic amino acids. In agreement with these findings, Emouset al. (2019) emphasized that decreasing the CP level in broiler diets not only reduces ammonia emissions but also mitigates total nitrogen losses from litter and manure. The cumulative evidence emphasizes the importance of dietary management strategies in poultry farming to minimize environmental impacts and optimize production efficiency.

Production performance

A significant treatment effect was observed in the average body weight gain (BWG) of broiler chickens over a four-week period (Table 4). The birds provided with a diet containing high crude protein (CP) showed better results than those in the other groups. These findings align with prior studies by Hernandez et al. (2012), Folorunso et al. (2014), and Liu et al. (2017). Allameh and Toghyani (2019) observed a significant decrease in broiler chicken weight with a reduction in dietary crude protein (CP) from 20.4% to 17.9%, further noting negative effects on daily weight gain when the dietary CP met only 85% of the requirements. Similarly, Hilliaret al. (2020) and Macellineet al. (2020) concluded that birds fed low-protein diets exhibited lower body weight gain compared to those on high-protein diets.

A significant treatment effect was observed in the average feed intake of broiler chickens over four weeks, with the high CP group consuming more feed than the other treatment groups. Ferguson et al. (1998) observed that increasing the crude protein (CP) content of diets from 22% to 26.4% did not result in significant changes in feed intake or body weight gain (BWG). Interestingly, they found that the feed-to-gain ratio decreased as the CP level in the diet was reduced, indicating a potential compromise between protein efficiency and growth performance. Consistent with these findings, Si et al. (2004), Waldroup et al. (2005), and Yamazaki et al. (2006) reported similar results, confirming that a reduction in CP during the grower period might lead to a reduction in feed intake.

Lower FCR means higher the performance. Lower FCR, which indicates that taking lower feed intake and their body weight gain is higher. The feed conversion ratios were not significant ($P > 0.05$) in any treatment group. The feed supplemented with high CP in the T_1 group resulted in a higher FCR while it was comparatively similar in the T_2 group and T_3 group respectively. These findings contrast with those of Malamoet al. (2013), indicating a significant influence ($P < 0.05$) of a high CP diet on feed conversion ratio. Conversely, Mamdouhet al. (2020) reported that chickens fed a diet comprising 100% required CP supplemented with citric acid demonstrated noteworthy enhancements in feed conversion ratio.

Carcass weight

The carcass weight in the high protein (CP) group is superior to that of the other groups. The results indicate that the treatments had significant effects ($P < 0.05$) on dressing percentage and the weights of dressed wings, breast, back,

and drumsticks, but no significant effects on thigh weight. The current findings align with previous research by Malomoet al. (2013), who reported higher carcass and breast meat yields in broiler carcasses fed a high-protein diet. These results are also consistent with the study by Saleh et al. (2021), where dressing percentages were significantly affected ($P < 0.05$) by reducing dietary protein levels.

Blood parameter

Blood parameters resulting from the various experimental diets are presented in Tables 6. The blood parameters, including hemoglobin, WBC, RBC, neutrophils, and lymphocytes, were affected ($P < 0.05$) in different treatment groups. Zhao et al. (2009) demonstrated that alterations in the crude protein (CP) content of broiler chicken diets can significantly influence various blood parameters. This finding underscores the importance of dietary composition in modulating physiological responses in poultry, highlighting the need for precise nutritional strategies to optimize health and performance. However, platelets, monocytes, eosinophils, PCV (packed cell volume), and MCV (mean corpuscular volume) were not significantly different ($P > 0.05$). These results align with the findings of Swennen et al. (2005), Kamran et al. (2010), and Mohamed et al. (2012), who concluded that the level of dietary protein does not alter glucose concentration. However, the results contrast with those of Alamet al. (2004) and Mohamed et al. (2012), who indicated that the dietary protein level had no significant effect on the hemoglobin concentration of broilers. Additionally, Mohamed et al. (2012) reported that dietary protein supplementation specifically led to an increase in the globin section of hemoglobin, while it had no noticeable impact on the haem portion. This finding suggests that protein intake may influence some parts of hemoglobin production without changing its overall structure.

Economic impact

The cost of different treatment groups is presented in Table 7. The total expenditure (Tk) per bird was higher in the high CP treated groups and lower in the low CP treated group. Feed costs were comparatively higher in the high-CP-treated group. Profit per bird was higher in the treatment groups but not significantly different. Benefit-Cost Ratio (BCR) was also statistically higher ($P < 0.05$) in the high CP group compared with the low CP group. These results are in agreement with the findings of Kumari et al. (2016), who reported that the application of high dietary protein in economic analysis revealed that it could be a cost-effective management practice to improve the shed environment and in turn the performance of broiler chicks.

Table 1:- Composition of the experimental diet (Broiler starter diets).

Diets (CP%) / Phases	*RPL (23%)	RPL (21%)	RPL (19%)
Ingredients%			
Corn	51.0	55.2	58.9
Soybean meal 44%	24.0	19.4	16.4
Rice polish	8.0	10.0	11.7
Soybean oil	3.0	1.6	1.0
Fish meal	10.0	9.1	7.4
Di-Ca phosphate	1.3	1.8	1.8
Limestone	1.31	1.51	1.41
NaCL	0.5	0.5	0.5
Choline chloride	0.1	0.1	0.1
**Vitamin & Mineral Premix	0.5	0.5	0.5
DL-Methionine	0.1	0.1	0.1
Lysine sulfate 70%	0.09	0.09	0.09
Threonine	0.1	0.1	0.1
Total	100.00	100.00	100.00
Calculated Values (%)			
ME (Kcal/Kg)	3001.39	3003.63	3004.02
Crude protein	23.03	21.02	19.06
Calcium	1.002	1.008	1.008
Inorg. Phosphorus	0.470	0.490	0.490

*Recommended protein level (RPL) ** Supplied per kilogram of diet: Vitamin A (Retinol), 10000 IU; Vitamin D₃ (Cholecalciferol), 1500 IU; Vitamin E (α -tocopheryl acetate), 10 mg; Vitamin K₃ (Menadione), 2.0 mg; Vitamin B₁ (Thiamin), 1.0 mg; Vitamin B₂ (Riboflavin), 5.0 mg; Vitamin B₆ (Pyridoxine), 1.5 mg; Vitamin B₁₂

(Cyanocobalamin), 0.03 mg; Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 2.0 mg; Biotin, 0.05 mg; Cu, 15 mg; I, 2.0 mg; Fe, 30 mg; Zn, 50 mg; Mn, 60 mg; Co, 0.1 mg; and Se, 0.15 mg.

Table 2:- Composition of the experimental diet (Broiler grower diets).

Diets (CP%) / Phases	*RPL (22%)	RPL (20%)	RPL (18%)
Ingredients%			
Corn	57.5	58.5	63.3
Soybean meal 44%	19.5	18.3	16.0
Rice polish	5.0	8.0	7.2
Soybean oil	2.8	2.8	2.5
Fish meal	11.1	8.3	6.5
Di-Ca phosphate	1.4	1.4	1.6
Limestone	1.31	1.31	1.51
NaCL	0.5	0.5	0.5
Choline chloride	0.1	0.1	0.1
Vitamin & Mineral Premix **	0.5	0.5	0.5
DL-Methionine	0.1	0.1	0.1
Lysine sulfate 70%	0.09	0.09	0.09
Threonine	0.1	0.1	0.1
Total	100	100	100
Calculated Values (%)			
ME (Kcal/Kg)	3101.23	3100.88	3102.48
Crude protein	22.01	20.00	18.04
Calcium	1.003	1.003	1.006
Inorg. Phosphorus	0.450	0.460	0.480

*Recommended protein level (RPL) **Supplied per kilogram of diet: Vitamin A (Retinol), 10000 IU; Vitamin D₃ (Cholecalciferol), 1500 IU; Vitamin E (α -tocopheryl acetate), 10 mg; Vitamin K₃ (Menadione), 2.0 mg; Vitamin B₁ (Thiamin), 1.0 mg; Vitamin B₂ (Riboflavin), 5.0 mg; Vitamin B₆ (Pyridoxine), 1.5 mg; Vitamin B₁₂ (Cyanocobalamin), 0.03 mg; Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 2.0 mg; Biotin, 0.05 mg; Cu, 15 mg; I, 2.0 mg; Fe, 30 mg; Zn, 50 mg; Mn, 60 mg; Co, 0.1 mg; and Se, 0.15 mg.

Table 3:- Effect of dietary crude protein on weekly ammonia gas emission (ppm) in broiler chickens.

Treatments	1 st week	2 nd week	3 rd week	4 th week
T ₁	3.2 ^a ±0.00	6.23 ^a ±0.03	8.80 ^a ±0.05	11.63 ^a ±0.17
T ₂	3.2 ^a ±0.00	5.80 ^b ±0.05	8.30 ^b ±0.11	10.36 ^b ±0.26
T ₃	3.2 ^a ±0.00	3.46 ^c ±0.03	5.30 ^c ±0.15	6.53 ^c ±0.14
Mean±SE	3.2±0.00	5.16±0.43	7.46±0.54	9.51±0.77
Significance	NS	*	*	*

Here, T₁ = (High CP), T₂ = (Medium CP), T₃ = (Low CP), Values are Mean ± SE (n=9). ^{a-c} Within a column, means sharing different superscripts differ significantly (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly. SE = Standard Error. NS = Non-Significant (P>0.05) * = Significant (P<0.05).

Table 4:- Effect of dietary crude protein on production performance of broiler chickens.

Treatments	Average Live Weight (g/bird)	Average Body Weight Gain (g/bird)	Average Feed Intake (g/bird)	FCR
T ₁	1927.34 ^a ±33.75	1885.34 ^a ±33.75	2560.57 ^a ±47.32	1.35±0.02
T ₂	1835.80 ^b ±15.83	1793.98 ^b ±15.92	2374.71 ^b ±22.43	1.32±0.02
T ₃	1779.20 ^b ±18.91	1737.20 ^b ±18.91	2317.30 ^b ±24.70	1.33±0.01
Mean±SE	1847.44 ±24.72	1805.50±24.70	2417.53±40.33	1.33±0.01
Significance	*	*	*	NS

Here, T₁ = (High CP), T₂ = (Medium CP), T₃ = (Low CP), Values are Mean ± SE (n=9). ^{a-b} Within a column, means sharing different superscripts differ significantly (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly. SE = Standard Error, NS= Non-Significant (P>0.05), * = Significant (P<0.05).

Table 5:- Effect of dietary crude protein on carcass weight (g) of broiler chickens.

Treatments	Dressing Percentage	Breast	Thigh	Wing	Back	Drumstick
T ₁	70.81 ^a ±0.76	564 ^a ±7.54	193.33 ^a ±4.97	89.66 ^a ±3.17	228.00 ^a ±9.23	184.33 ^a ±4.40
T ₂	67.10 ^b ±0.70	527 ^b ±4.35	180.00 ^a ±6.08	86.33 ^{ab} ±3.48	207.66 ^{ab} ±3.38	170.00 ^{ab} ±6.08
T ₃	66.52 ^b ±0.38	518 ^b ±5.50	174.33 ^a ±8.35	77.00 ^b ±2.88	199.00 ^b ±2.64	157.00 ^b ±4.16
Mean±SE	68.14±0.74	536.33±7.64	182.00±4.34	84.33±2.47	211.55±5.20	170.44±4.66
Significance	*	*	NS	*	*	*

Here, T₁ = (High CP), T₂ = (Medium CP), T₃ = (Low CP), Values are Mean ± SE (n=9). ^{a-b} Within a Column, means sharing different superscripts differ significantly (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly. SE = Standard Error, NS = Non-Significant (P>0.05), * = Significant (P<0.05).

Table 6:-Effect of dietary crude protein on blood parameter of broiler chickens.

Parameter	Treatments			Mean±SE	Significance
	T ₁	T ₂	T ₃		
Hemoglobin (g/dl)	9.61 ^a ±1.54	12.55 ^{ab} ±0.99	8.66 ^b ±0.58	10.27±0.80	*
WBC (×10 ⁹ /L)	15.00 ^a ±1.10	18.0 ^{ab} ±4.70	14.2 ^b ±1.30	15.70±7.7	*
RBC (×10 ¹² /L)	4.64 ^a ±0.50	5.13 ^{ab} ±0.23	3.79 ^b ±0.25	4.52±0.26	*
Platelet (%)	38.50±4.30	42.30±6.80	46.50±7.30	42.4±3.30	NS
Neutrophil (%)	77.33 ^a ±1.20	76.33 ^a ±2.60	83.00 ^b ±1.00	78.88±1.35	*
Lymphocyte (%)	17.00 ^a ±1.73	17.00 ^a ±2.08	11.00 ^b ±0.00	15.00±1.26	*
Monocyte (%)	1.33 ^a ±0.33	2.00 ^a ±0.57	1.66 ^a ±0.66	1.66±0.28	NS
Eosinophil (%)	4.33 ^a ±0.88	4.66 ^a ±0.33	4.33 ^a ±1.45	4.44±0.50	NS
PCV (%)	30.58 ^a ±5.78	38.87 ^a ±2.92	26.57 ^a ±1.75	32.01±2.65	NS
MCV(fl)	82.83 ^a ±4.64	87.46 ^a ±3.91	74.81 ^a ±2.01	81.70±2.61	NS

Here, T₁ = (High CP), T₂ = (Medium CP), T₃ = (Low CP), Values are Mean ± SE (n=9). ^{a-b} Within a Column, means sharing different superscripts differ significantly (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly. SE = Standard Error, NS = Non-Significant (P > 0.05), * = Significant (P<0.05).

Table 7:- Effect of different levels of crude protein on the economic aspects of broiler management practices.

Treatments	Total expenditure	Receipt per bird	Profit per bird	Benefit cost ratio
T ₁	199.05 ^a ±2.39	269.78 ^a ±4.70	70.73 ^a ±4.19	1.35 ^a ±0.02
T ₂	189.71 ^b ±1.13	256.99 ^b ±2.21	67.27 ^a ±2.99	1.35 ^a ±0.01
T ₃	186.83 ^b ±1.22	249.01 ^b ±2.61	62.18 ^a ±3.83	1.33 ^a ±0.02
Mean± SE	191.86±2.02	258.59±3.45	66.72±2.23	1.34±0.01
Significance	*	*	NS	NS

Here, T₁ = (High CP), T₂ = (Medium CP), T₃ = (Low CP), Values are Mean ± SE (n=9). ^{a-b} Within a Column, means sharing different superscripts differ significantly (P<0.05). Mean within same superscripts don't differ (P>0.05) significantly. SE = Standard Error, NS = Non-Significant (P>0.05), * = Significant (P<0.05).

Conclusion:-

Adjusting and stabilizing the dietary crude protein level in broilers serves as a crucial strategy for mitigating ammonia emissions; however, there is a limit to the reduction of crude protein without adverse effects on bird performance and nitrogen economy. This study demonstrates that a higher crude protein content (23% in the starter phase and 22% in the grower phase) contributes to better production performance, improved carcass quality, and increased economic benefits in broiler rearing. Despite its production benefits, high-protein feed is cost-ineffective and leads to elevated ammonia emissions. Additionally, balancing protein levels with ammonia emissions and environmental impact is important for sustainable broiler production. Hence, further research is needed to check the amino acid profile to enhance performance with lower crude protein diets.

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Conflict of Interest

All authors declare that they do not have any conflicts of interests that could inappropriately influence this article.

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